ON THE DISTRIBUTION AND MODE OF OCCURRENCE IN THE UNITED STATES AND CANADA OF CLINOSTOMUM MARGINATUM, A TREMATODE PARASITIC IN FISH, FROGS AND BIRDS.

HENRY LESLIE OSBORN.

Contents.

1. Geographical Distribution of *Clinostomum marginatum*.

The fluke referred to in this paper was first noticed in this country in 1856 by Joseph Leidy, in the intestine of the pike of the Delaware and in cysts attached to the gills of the sun-fish (*Eupomotis vulgaris*) near the city of Philadelphia. Leidy applied the name *Clinostomum gracile* to it. This generic name after many years of non-use has come into current usage since the late revision of the Trematodes which has received such impetus from the work of Looss ('00) who recognizes this name and Braun ('00) who in a revision of the genus in 1900 recognizes eight species of the genus *Clinostomum*, among them *C. marginatum*. In 1879 R. Ramsey Wright found cysts attached to the gills, branchiostegal membranes and pectoral fins of the yellow perch (*Perca flavescens*) at Toronto, Ontario, which he recognized as being identical with the *Clinostomum gracile* of Leidy and called *Distomum gracile*. His observation extended the geographical range of the species to the St. Lawrence River system. In the same paper Professor Wright reported the finding of *D. gracile* in the bittern (*Botaurus minor*) a fish-eating bird which was the first information as to the definitive host of the worm. Looss in 1885 published an account of the structure of a fluke which had been found encysted in the muscle of a silurid fish from Porto Rico. He gave to this fluke the name *Distomum reticulatum* in allusion to the very peculiar excretory collecting apparatus. Subsequent writers, including Looss himself (Looss '99), have referred this worm to the species *Clinostomum marginatum*, and while there are certain points in which it differs from *C. marginatum* we may for the present so recognize it. This observation however takes the fluke a long way out of the region in which we otherwise know the animal and seriously embarrasses the attempt to deal with the geographical range of the species. In 1888 Leidy reported a fluke from the striped bass (*Roccus lineatus*) which he designated *Distomum galactosomum*. His account of the organization of this form touches on the form of the oral end of the body and the network of collecting vessels in the body-wall, two features of such peculiarity that we cannot doubt but that the subject of his study was none other than *Clinostomum marginatum*, which he had described in 1856 and evidently forgotten. His account located the worm in a marine host for the first time, unless Looss' silurid was marine.

In 1895 MacCallum ('99), of the University of Toronto, found a trematode encysted in the muscles of the frog which he regarded as identical with the ones which Wright had found in the fish and heron and referred to the *D. gracile* of Leidy. In his paper of 1899 MacCallum calls the worm *C. heterostomum*.

His description, while differing in some respects from my material, leaves no doubt but that our material is identical. I shall adopt the name *C. marginatum* in place of the name used by MacCallum following the lead of Braun ('00) in his revision of the group. He also found the same species in the throat of *Ardea herodias* at Danville, Ontario.

In 1898 Linton reported and figured this species under the name of *D. gracile* from the sun-fishes (*Eupomotis pallidus* and *Chænobryttus gulosus*) of Kansas City, Mo. This observation extended the range of the worm to a new river system, the Missisippi and gave us the most western point of its distribution. In 1900 specimens of this fluke were found by myself at Nebish, Mich., encysted in the muscular tissues of the small-mouthed black-bass (*Micropterus dolomieu*), also, though less frequently, in the muscles of the yellow perch (*Perca flavescens*). They were also found later in the same year in the throat of the little blue heron (*Ardea herodias*). Nebish is a small camping place situated on the St. Mary's River, about twenty-five miles below the outlet of Lake Superior. A new locality was thus added to the distribution of the worm though one in the same river system as the Canadian ones.

In 1903 I received, through the kindness of Professor Linton, some pieces of the small-mouthed black bass infected with this parasite. The fish had been taken by the Rev. J. H. Young at Troy, Ohio, on the Miami River, a tributary of the Ohio, and part of the Mississippi system. Some of the worms were encysted in muscle, but others were located in the skin on the internal aspect of the branchiostegal membranes. Their location is shown in Fig. 1 of this article.

In the same year my attention was called by Dr. W. S. Nickeson, of the University of Minnesota, to certain flukes which he had found in frogs, and they were at once recognized as specimens of this species. At about the same time I began to notice them in the frogs of this region brought to the laboratory for use as biological material. The details of the occurrence of the fluke in this region is still under investigation in connection with a study of the parasites of the frogs of this vicinity, so that a fuller account of this part of the subject is reserved for a later occasion. It is important, however, to place on record the occurrence of the fluke in this region, as it extends the range of the parasite considerably beyond formerly recorded limits.

The latest appearance of this form in literature is the record of its recognition in the yellow perch of the Montreal markets by Stafford in 1904, where it is given Leidy's designation *Clinostomum gracile.*

The foregoing facts show that this species is very widely distributed in this country, having been recognized at Kansas City on the West and at St. Paul, Michigan, and as far east as Montreal. It has been seen as far north as St. Paul and as far south as Philadelphia and in Ohio in the center of this territory. The occurrence at Porto Rico is anomalous and cannot be considered until more information is forthcoming. In view of the wide distribution and large size of this species, it seems strange that we are not in possession of a particle of information regarding the early stages of its life-history. The worm is fully developed when it is encountered in the fish or frog and all that is needed for its complete maturity is that the vitellaria shall become active, which they do as soon as the parasite reaches the heron. The details of structure of the worms of the bass have been determined both in total preparations and in serial sections. In the smallest individuals the entire organization is developed. It follows from this fact that there are three hosts involved in the life-history of this species of which the fish or frog is the second and the heron the final one, the first being unknown. The rôle of the fish or frog does not appear to be one of importance from the view point of the ontogeny, but seems merely to be for the purpose of getting the worm to the heron. It thus would appear that the primary host is not one which serves as food to the heron but does serve as food for the bass. This would fit the case of insects as well as any group of invertebrates.

In view of the fact that this parasite infects the edible portion of such important game and food fishes a knowledge of its life history is particularly desirable. While the parasite is fortunately one which is not injurious to the human system, at the same time its presence in the bass and perch unfit them for the table. It is clear that the worm is already widely distributed and is a menace to fish-culture, which at any time may become of great importance. It seems likely that if one were able to take up the problem of this life-history under favorable conditions for instance at some small body of water where the infected fish were abundant and to follow it up at various seasons the missing data could be obtained.

The data given in the foregoing paragraphs are summarized in the following table.

Date.	Observer.	Name Used.	Host.	Seat of Infection.	Locality.
1809	Rudolphi.	*Distomum marginatum.*			
1856	Leidy.	*Clinostomum gracile.*	*Esox.*	intestine.	Philadelphia.
1879	Wright.	*Distomum gracile.*	*Perca flavescens.*	branchiostegal membranes.	Toronto, Ont.
1879	Wright.	*Distomum heterostomum.*	*Botaurus minor.*	mouth.	Toronto, Ont.
1885	Looss	*Distomumi reticulatum.*	Silurid fish.	muscle encysted.	Porto Rico.
1888	Leidy.	*Distomum galactosomum.*	*Roccus lineatus*	——	Philadelphia.
1895	MacCallum.	*Distomum gracile.*	Frog.	muscle.	Toronto, Ont.
1897	MacCallum.	*Distomum heterostomum.*	*Ardea herodias.*	mouth.	Toronto, Ont.
1898	Linton	*Distomum gracile.*	*Eupomotis pallidus.*	pectoral fin.	Kansas City, Mo.
1898	Linton.	*Distomum gracile.*	*Chænobryttus gulosus*	roof of mouth.	Kansas City, Mo.
1901	Osborn.	*Clinostomum marginatum.*	*Micropterus dolomieu.*	muscle.	Nebish, Mich.
1901	Osborn.	*Clinostomum marginatum.*	*Perca flavescens.*	muscle.	Nebish, Mich.
1903	Osborn.	*Clinostomum marginatum.*	*Rana virescens.*	cœlom wall.	St. Paul, Mn.
1903	Young,	*Clinostomum marginatum.*	*Micropterus dolomieu.*	branchiostegal membranes.	Troy, Ohio.
1903	Young.	*Clinostomum marginatum.*	*Micropterus dolomieu.*	muscle.	
1904	Stafford.	*Clinostomum gracile.*	*Perca flavescens.*	gills.	Montreal, Ont.

2. Mode of Occurrence in the Black Bass and Perch, at Nebish, Mich.

The observations now to be described were made at Nebish in 1901 and repeated the following year. Nebish is a small settlement on the American side of the St. Mary's River about midway between Lake Superior and Lake Huron. At that time bass and perch were fairly numerous, beginning in the early part of September and the last of August. As they were not caught much earlier it was the local opinion that the fish migrate to the grounds at Nebish from elsewhere, and this is borne out by the fact that of late years, as a consequence of the work done in improving the channel of the river for navigation, the bass have ceased to come in the fall and the "fishing" is destroyed. I examined as many of the various kinds of fish and invertebrates as possible during my short stay at Nebish in hope of getting some information which would lead up to the discovery of the first host. The only forms on which I recognized the parasites were the bass and the perch. Other fishes which were investigated with wholly negative results were the grass-pike, which is very often caught there, and the sun fish, *Eupomotis*—also very common. Various gasteropods and insects were examined but without any result. I have thus far not been able to obtain any clue to the source of infection of the fish.

The percentage of infected individuals found among the bass was very great. I do not happen to have any statistical data bearing on this point, but the parasite was found in nearly every bass submitted to examination, while in the perch it was much more rare. The cysts were very easily seen, being large, opaque and very creamy white, in marked contrast with the darker semi-translucent muscular tissue in which they lie imbedded. When the fish were skinned in preparing them for cooking the cysts walls were often torn open and the conspicuous worm seen moving on the surface of the meat. The cysts were found in all parts of the lateral muscles, deep and superficial, dorsal and ventral and headwards and tailwards. There was no evidence from their location bearing on the question of the mode of entry which had been adopted by the worms, but the observations of various others who have reported the worm from the gills, roof of mouth, branchiostegal membranes and pectoral fin would indicate that the worm enters the fish in the head region. If so it is difficult to imagine how they reach the places where we find the cysts if they are as large as we find them when they enter. On the other hand, if they enter as small and immature stages we should surely be able to find some evidence of it. Some of the worms should be less fully developed than others, or some of the waste products of the chemical processes involved in growth should be recognizable. So that we are unable to find a solution to this problem with the data at present available.

The number of cysts in single individuals varied greatly. The minimum number found was seven and the maximum number was more than one hundred. The appearance of one of the cysts in situ in the muscle is shown in Fig. 2. They are oval or spherical. They are smaller than those reported by Looss ('85) from the fish which he examined, being 1.3–3 mm. long as compared with 3.5–4 mm. in length according to Looss.

The cysts occupy a space in the endomysium among the fibers of the muscle of the host. As we do not possess many detailed accounts of the structure of trematode cysts or their relation to the host tissues, an account of the facts found in this case may be of some interest. The following methods were employed. The cyst was carefully separated by teasing away the surrounding muscle fibers of a piece which had been fixed and hardened in suitable reagents. The cyst was then cut open and the enclosed worm removed, after which pieces of the wall were submitted to the action of various staining reagents and mounted for microscopical study. In other cases the muscle and enclosed cyst was sectioned serially. In such a series we have transverse sections of the wall and the surrounding muscle tissue, and at the ends of the series there are tangential sections which can be compared with the flat preparation just mentioned. Figs. 3 and 4 are low- and highpower views of such sections, and they show the structural factors involved. The muscle fibers are bent around the cyst as seen on the sides of the section; those in the center which seem to end abruptly at the surface of the cyst do not in reality do so, but are cut off as they run out of the plane of the section. The worm wholly fills the cavity of the cyst, the very small space which is seen in the figure being easily accounted for by the shrinkage of the worm. The structure of the cyst is seen in Fig. 4 to be merely a membrane produced by the condensation of fibrous tissue. There are the usual wavy fibers, thinning out as they reach the endomysium, and continuous with the fibers which fill in the spaces between the muscle fibers, and between the fibers the customary flattened connective tissue nuclei. The flat preparations and tangential sections show very clearly that the cyst is supplied with a capillary network derived from the vascular supply of the muscle, and these capillaries and their contained blood corpuscles can be recognized in the section of the wall as shown in Fig. 4. Looss states ('85) that his observations led him to conclude that the cyst is produced by the host and then lined by a second envelope produced by the worm itself. "Darunter befinded sich eine zweite Hulle, die, anscheinend ein erhärtetes Secret, von dem

Wurme um sich erzeugt wurde, und in dieser liegt eingerollt und zusammengeschlagen das Thier selbst." As to this inner lining found by Looss no traces of it are found in my material, and the cyst is wholly a product of activities on the part of the host tissues. The cyst may be looked upon as a defense made by the host against the parasite. It would be interesting to know the nature of the stimuli which have caused this reaction on the part of the supporting tissues of the muscle whether they are merely mechanical and acting in the form of pressure or whether they are chemical or both. The excessive formation of connective tissue and the growth of a definite and extensive capillary network have resulted from the presence of the worm. In the somewhat analogous case of the cavities produced in oak leaves by the sting of certain hymenoptera for the purpose of housing their larvæ, the normal growth of the leaf is turned aside sufficiently to produce cavities for the larva, walled with a material produced by the leaf from its own substance. In that case the stimulus would seem to be chemical. Whether it is so in this case cannot be determined until we are in possession of more facts connected with the introduction of the parasite to the tissue of the fish.

In the bass, as already stated, the worm entirely fills the cavity of the cyst. In order to determine its position within, several fixed and hardened cysts were carefully dissected out of their place in the muscle and the wall carefully removed under a dissecting microscope. The worms thus found bent and cramped within were macerated for an hour or less in tepid water till they became flexible and could be uncoiled as shown on Fig. 5. They were thus found to be bent twice, both times with the ventral surface outward, first on the level of the meeting of the first and second body thirds, and again on the level of the second and third body thirds. The first of the bends is in the median plane of the body and brings the dorsal surfaces in contact; the ventral sucker is thus exposed and serves to identify the surface posiively. In making the second bend the body does not bend dorsally at once but first there is a twist which brings the dorsal side over the edges of the first and second thirds of the body and then the dorsal surface of the last body third laps over these edges. The worms and cysts in the frog are very different from this. They will receive attention later.

3. Behavior of Worms Liberated from the Bass Cyst.

The living worms are easily liberated artificially by cutting into the cyst wall with a sharp instrument. As soon as a small opening has been made the worm begins to extrude through it almost as if it had been confined under some degree of pressure. It continues to make active movements of extension and contraction which soon result in freeing it from the cyst. It continues to move actively after gaining its freedom. In the course of nature the worm on emerging from the fish cyst would find itself in the stomach or intestine of a fish-eating bird; those which were removed artificially were received and kept in shallow vessels of fresh water where their activities could be watched. The surroundings in which they thus found themselves were quite unnatural and perhaps it was for this reason that they were so very active. The movements did not result in locomotion, though they produced constant changes in the form of the body, but the worm did not progress. Some trematodes adhere very forcibly by their suckers to any object with which they come in contact but there was no attempt made on the part of these to use the suckers for that purpose. Some of the various movements were so irregular and indefinite as to preclude the possibility of grouping them under any general head, but there were others which were definite and constant enough to be classed under either of two groups which I have called "poses" from the fact that in each case a series of movements took place culminating in a certain bodily form or attitude which was only momentarily maintained and after which the body relaxed and fell back to its ordinary resting posture. These two poses were seen often enough to justify the conviction that they are a constant feature of the behavior of the worm and so merit a detailed description. The two poses will be designated the "suctorial pose" and the "swimming pose." Sometimes the same pose is repeated several times in succession, at other times the two alternate irregularly.

The suctorial pose is represented in Fig. 6 as seen in the living animal and in Fig. 7, a view of a sagittal section of the anterior end of the body of an animal which happened to be caught in this pose at the moment of fixation. The region of the body chiefly active in the production of this pose is the part anterior to the ventral sucker. Ordinarily this part of the body has the shape of an obliquely truncated cylinder. In assuming this pose the worm draws the end of the body back into the interior, the side walls being thicker and acting as the rim of a sucker projecting considerably beyond the level of the center. This inversion of the oral end of the body is brought about by the contraction of fibres of the parenchyma muscle system which run longitudinally in the body. Some of these fibres are shown in Fig. 7. Their contraction pulls the center of the oral end back and the margin is left projecting. If this action were to take place at a moment when the oral end was in contact with a soft surface, for example, such a surface as the mucous coat of the stomach or throat of the heron, then the soft substance of the host would be sucked into the cavity of this sucker and a powerful adhesion of the parasite to the host would result. A worm liberated naturally from the cyst would meet conditions which would furnish responses to this movement, so that instead of being merely a momentary pose it would be useful and so continued. On the other hand, in the case of the artificially liberated worm such stimuli being absent the worm returns to its customary form. The sucker thus formed is additional to the two usual trematode suckers. We may suppose that in as large a parasite as *Clinostomum* and one which lives in such an exposed place as the gullet where large masses of food are pressed against it additional adhesion would be needed to protect it against being dislodged by the pressure of the food as it is being swallowed. This striking movement had thus evidently a purposive character, as can be seen if we take into account the environment for which it has been developed.

The "swimming pose" is shown in Fig. 9. In this case the body is made broader and flatter than usual, the ventral surface becoming somewhat concave. The margins of the posterior part of the body are reduced to fine sharp lines like fins which do not extend into the front part of the animal but crossing it ventrally converge toward the ventral sucker. The line shown in the figure is not visible in any specimens of the worm after preservation and is only a momentary structure. The flattened form of this pose too is only momentary, and no preserved specimens show it. In all cross sections of the worm the body is elliptical and has a thickness of nearly half its breadth.

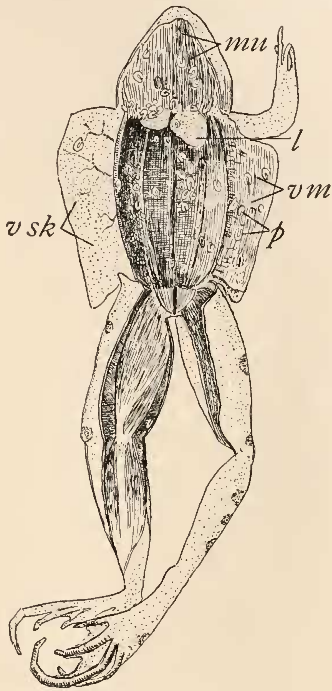
The transverse parenchyma muscles are the ones which are used in the swimming pose, the longitudinal muslces being at rest. Alternate contractions of the transverse and longitudinal muscles would throw the animal first into one, then into the other of these positions. The observation of certain leeches furnished the suggestion for calling this the swimming pose. In the leeches the margins of the body are thinned in this way and reduced to a thin lateral fin and the worms swim rapidly through the water. In the specimens of *Clinistomum* which were under observation no vibrations of the body were made and the pose was not turned to any account. It is possible that in nature this body form would be adapted to progression through the chyle and chyme of the bird and would be the means by which the worm reaches the throat toward which it is travelling from the stomach or intestine. It would be very interesting to experiment with these worms by removing them from cysts and placing them on surfaces as much like the avian mucous membranes as possible so as to ascertain whether these poses are as adaptive as they seem to be.

4. On the Cysts in the Frog.

The first mention of the frog as a host of *Clinostomum* is by MacCallum ('99), who reports it from the pectoral muscles of frogs found in Ontario. We have been finding it now for several years in the frogs caught in the vicinity of St. Paul which are used in our biological courses.[1] The mode of infection of the frog is different from that of the bass. The cysts, instead of being located in the muscular tissue, are seldom found there, but are in the peritoneal lining of the cœlom or in the lymph spaces between the skin and the muscles of various parts. Text-figure 1 is drawn from the most considerably infected specimen which I have met. The cysts are located in the cœlomic cavity;

[1] It is most probable that frogs and fishes in many parts of this country are infected by this parasite. Any information as to localities elsewhere where it has been noticed would be very gratefully received by the writer. A study of the relation of the worm to the frog is now in progress, and it is hoped that clues to the early history of the worm may be obtained through its connection with the frog.

especially in the region dorsal to the heart. They are found here most frequently. They are also found in the ventral wall of the cœlomic cavity, in the floor of the mouth between the muscles and the skin and even in the leg between the muscle and the skin. In no case have the cysts been found in the frogs of this region in the interior of muscular tissue surrounded by the fibers as they are so characteristically found in the fish. I have as yet not been able to find any reason for this difference in the location of the cysts with regard to the host, but the position of the worm within the cyst is considerably affected, as will be explained fully in a moment.



View of ventral surface after the removal of the cœlomic viscera, showing the numerous encysted flukes in place. *l*, lung; *mu*, muscles of floor of mouth; *p*, flukes in place; *v*, ventral muscles of cœlomic cavity; *v sk*, skin of ventral cœlomic wall. ×2/3 natural size.

The ratio of infection of the frog does not appear to be as high as it is in the fishes studied at Nebish. The percentage of frogs not infected is very high, indeed a large number can be gone through often without any cysts being found. The number of cysts in a single individual is usually not large; the frog figured is quite exceptional in that respect. Data for an exact statement on these points are not at present worked out.

The relation of the worm to the cyst and to the underlying muscle of the body wall is shown in Fig. 9. The muscle fibers run along below the cyst, their course entirely uninfluenced by the presence of the cyst. A comparison of Fig. 9 with Fig. 2 shows the difference of habit in this respect at a glance. The preparation from which Fig. 9 was drawn shows five cysts in an area half an inch wide by a quarter of an inch across. It is removed from the right half of the body wall of the frog shown on page 361. The whole piece was stained and mounted in balsam and one of the cysts drawn in Fig. 9. The cyst is much larger than the worm and there are spaces within unoccupied by the worm. The worm in most cases is bent once, only the ventral surface being turned outward. In some cases the bend is not in the center, and then the longer end may be bent slightly as in the one shown in Fig. 9. The frog cysts show the presence of a rich network of capillaries spread out over their surface. These are derived from vessels which come from large and conspicuous vessels running in the space between the peritoneum and the muscle layer. The ordinary surface of the peritoneum does not possess these capillaries, which are evidently a growth developed as a result of the presence of the cysts.

The specimens of *Clinostomum* seen in the frogs are all virtually fully matured, having the full size of the heron specimens. Their inner organization too is identical in appearance with that of specimens from the fish, and excepting as to the vitellaria with that of the heron. There is thus no room for an hypothesis as to the frog being the first host and the medium by which the fish is reached, which hypothesis we might be tempted to frame, knowing that the frog is one of the foods of the bass, and other predaceous fishes. We are thus left to suppose a first host, possibly an invertebrate, followed by a second intermediate host, the fish which serves as a medium of transmission from the unknown first host to the heron. The case of a second host which serves to transmit a parasite from a first host where it develops to a final host where it matures and where sexual reproduction takes place has been recognized for a number of trematodes. A list containing 28 species is given by Braun (93, pp. 864–866), in which this species is not included. In some of these the frog or one of the fishes serves as the medium by which the final host is reached.

5. On the Occurrence of the Worm in the Heron.

A single specimen of the heron sent me by express from Nebish in October happened to be infected with this parasite and furnishes all that I have myself observed on this point. The bird had been shot and had been dead a day or two before it came into my possession. The worms were still alive and suitable for fixation and preservation for histological study. The worms were found in considerable numbers adhering to the wall of the throat by means of the anterior end used as a sucker in the manner referred to above. On being removed they were found to be mobile but no data as to their movements were recorded. The stomach and intestine of the heron were carefully examined to ascertain whether those organs were infected or not. No worms were found there. The stomach however contained the remains of the bodies of five fishes in various stages of digestion all of which were too decomposed for recognition with entire certainty but their elongate shape, their size and the fauna of the region made it virtually certain that they were sepcimens of the yellow perch. This observation furnishes the evidence of a connection between the fish and the final host. The liberation of the worms in the stomach and their migration of the worms in the stomach and their migration and adhesion to the wall of the throat is of course the first event of their life in the avian host followed by the production of eggs and their passage to the uterus where we find them in great numbers in the specimens taken from the heron. It is not exactly clear why the eggs are not passed directly from the body of the heron after the manner general in the flukes. Here however there is a large uterus into which the eggs pass and where they accumulate, for reasons as yet unknown. The eggs when they are expelled from the fluke must pass down the alimentary canal of the heron and fall with the feces in the water or less probably on the ground, where they make their way to the first host. The eggs in the uterus show no signs of development so that the ontogeny remains for the present a wholly unknown quantity.

Summary.

1. The first host and early life-history of *C. marginatum* are entirely unknown.

2. It passes a period of quiescence encysted in the muscle tissue of various predacious food fishes and in the lymph-spaces of frogs, in various localities ranging from Missouri through Ohio to Pennsylvania, and north as far as Minnesota, Michigan and Ontario, Canada.

3. It finally reaches an avian host which is some fish-eating bird, such as the bittern or the heron.

4. The cyst in the fish is a connective tissue structure produced from the endomysium of the host with a special vascular equipment.

5. The living worms on being artificially liberated from the cysts perform characteristic movements adapted to finding locations in the final host.

Biological Laboratory,
Hamline University,
Saint Paul, Minn.,
March 17, 1911.

LITERATURE.

Braun, M.
'93 Bronn's Klassen u. Ordnungen. Bd. IV., pp. 306–924. Trematoden.

Braun, M.
'00 Die Arten der Gattung Clinostomum Leidy. Zool. Jhrb. Syst., XIV.

Leidy, Jos.
'85 A Synopsis of the Entozoa, etc., no. 31. Proc. Ac. Nat. Sci. Phila., pp. 42–58.
'87 Notes on some Parasitic Worms, Proc. Ac. Nat. Sci. Phila., p. 24.

Linton, Edwin.
'98 Notes on the Trematode Parasites of Fishes. Proc. U. S. Nat. Mus., XX., pp. 597–548.

Looss, A.
'85 Beit. zur Trematoden, Distomum palliatum n. s. u. D. reticulatum n. s. Zeitf. w. Zool., XLI.
'99 Weit. Beitr. z. Kenntn. der Trematoden Fauna Aegyptens. Zool. Jahrb. Syst., XII., pp. 521–748.

MacCallum, W. G.
'99 On the species Clinostomum heterostomum. Am. Jour. Morphol., XV., pp. 697–710.

Osborn, H. L.
'01 Structure of Clinostomum. Science, XIII., p. 378.

Stafford, J.
'94 Trematoda from Canadian Fishes. Zool. Anzeig., XXVII., pp. 681–694.

Wright, R. R.
'79 Contrib. to Americ. Helminthology, No. 1. Proc. Canad. Inst., I.

Explanation of Plate I.

Fig. 1. View of the inner aspect of the right half of the lower jaw of a specimen of the small-mouthed black bass, from Ohio; showing the white cysts of *C. marginatum* at the bases of the branchiostegal rays, and black spots of an unidentified nature. Natural size.

Fig. 2. A cyst of *C. marginatum* in place among the muscle fibers of the bass from Nebish, Mich., from a glycerine preparation after teasing off part of the muscular tissue.

Fig. 3. View of a section passing through a cyst in place in the muscle tissue of the bass, from Nebish, Mich. Camera lucida, ×24 diameters.

Fig. 4. View from a section of a cyst in a plane transverse to the muscle tissue, showing in detail the histological structure of the wall of the cyst; from bass from Nebish, Mich. Camera lucida. ×340 diameters.

Fig. 5. Drawn from a specimen of *C. marginatum*, which had been removed from the cyst after fixation in alcohol and relaxed by slight maceration; from bass from Nebish, Mich.

Fig. 6. Sketch of living animal in "suctorial pose" as seen from ventral side; from bass, Nebish, Mich.

Fig. 7. From a sagittal section of anterior end of worm which died in "suctorial pose," from heron, Nebish, Mich. Camera lucida, × 38 diameters.

Fig. 8. View of ventral surface of a living animal in the "swimming pose," from bass, Nebish, Mich.

Fig. 9. View of a portion of the muscles of the ventral cœlomic wall of frog, showing a cyst and enclosed worm. From specimen after clearing and mounting in balsam. St. Paul, Minn. Camera lucida, × 35 diameters.